Cytokine patterns in experimental schistosomiasis mansoni infected mice treated with silymarin

Nagwa Mostafa El-Sayed · Ghada Mahmoud Fathy ·
Sara Abdel-Rahman Abdel-Rahman ·
Mahmoud Abdel-Atei El-Shafei

Received: 23 September 2014 / Accepted: 16 October 2014 / Published online: 30 October 2014
© Indian Society for Parasitology 2014

Abstract The purpose of this study was to determine cytokine patterns in experimental schistosomiasis mansoni infected mice treated with silymarin. The study was conducted upon 100 mice that were divided into five groups; 20 each: uninfected control group, *Schistosoma mansoni* infected untreated mice (infected control), infected mice treated with praziquantel (PZQ), infected mice treated with silymarin and infected mice treated with both praziquantel and silymarin. 10 mice from each group were sacrificed at 10th and 18th weeks post infection respectively. Histopathological investigations were performed. Liver sections were stained with hematoxylin–eosin and Masson’s trichrome stain to evaluate changes of granuloma sizes and numbers. Serum levels of the cytokines (TNF- α , IFN- γ , IL-4 and TGF- β 1) were assessed in the sera of all groups by immunoassay. The measured levels of cytokines (IFN- γ , IL-4, TNF- α , TGF- β 1) were found to be significantly increased in infected mice compared to normal control. At the same time, treated groups with silymarin alone or combined with PZQ showed significant decrease in IL-4,

TNF- α and TGF- β 1 levels compared to infected control. On the other hand, there was a significant increase in IFN- γ level observed in all treated groups compared to infected control. In addition, the histopathological examination of the liver in the group treated with PZQ showed a reduction in the number of livers eggs granuloma at all periods of sacrifice compared with the infected untreated group. However, there was more decrease in granulomas diameter in both silymarin treated group or combined with PZQ at all periods of sacrifice when compared to infected untreated group. In conclusion; treatment with silymarin combined with PZQ in murine schistosomiasis could reduce hepatic fibrosis by their action on the production of pro-inflammatory cytokines.

Keywords *Schistosoma mansoni* · Liver fibrosis · Cytokines · Silymarin · Praziquantel

Introduction

Schistosomiasis caused by *Schistosoma mansoni* continues to be an important cause of parasitic morbidity and mortality worldwide and is the most common fibrotic disease to arise due to inflammation and deposition of scar tissue around the parasite eggs trapped in the liver (Burke et al. 2010). The formation of granulomas around schistosome eggs is mediated by CD4⁺ T cells sensitized to egg antigens (Williams et al. 2001). Several studies have furnished numerous insights into the cytokine cascade that controls the development of these lesions. *Schistosoma*-associated fibrosis is influenced by several counter regulatory cytokines, including both of type-1 (IFN- γ , IL-2, TNF- α) and type-2 T cell associated cytokines (IL-4, IL-5, IL-6, IL-9, IL-13) (Hoffmann et al. 2002). Decreased fibrosis was

N. M. El-Sayed (✉)
Parasitology Department, Research Institute of Ophthalmology,
Ministry of Scientific Research & Technology, Giza, Egypt
e-mail: nagelsaka@hotmail.com; nag.elsaka@yahoo.com

G. M. Fathy · S. A.-R. Abdel-Rahman
Parasitology Department, Faculty of Medicine, Zagazig
University, Zagazig, Egypt
e-mail: dghadaamts@yahoo.com

S. A.-R. Abdel-Rahman
e-mail: sarameram@yahoo.com

M. A.-A. El-Shafei
Clinical Pathology Department, Faculty of Medicine, Zagazig
University, Zagazig, Egypt
e-mail: Mahmoudelshafei16@yahoo.com

associated with a diminished type-2 response and increased type-1 cytokine production.

In the murine model of schistosomiasis, interleukin-4 (IL-4), IL-5, and IL-13 contribute to granuloma formation and the presence of eosinophils in these lesions (Chiaramonte et al. 2001). The production of IL-4 and IL-5 in the granuloma is stimulated by soluble egg antigens (Kresina et al. 1992); IL-5 is responsible for the generation of eosinophils and IL-4 for the IgE response. Tumor necrosis factor- α (TNF- α), a soluble inflammatory cytokine secreted by activated macrophages in response to parasitic soluble antigens, may participate in the granuloma formation and evolution of the fibrotic tissue process while interferon gamma (IFN- γ) has a protective effect on severe fibrosis of the liver (Booth et al. 2004, Henri et al. 2002).

Praziquantel (PZQ) is an isoquinoline derivative and is the drug of choice for all species of *Schistosoma* as an effective antischistosomal drug (Utzinger and Keiser 2004). It acts to reduce granuloma sizes (El-Ahwany et al. 2006) apparently due to reduction of cellular infiltrates. Although treatment with this drug is effective, but frequent schistosome reinfection occurs after treatment due to the relative resistance to schistosomicidal drugs (Silva et al. 2003). At the same time, it is preferable to develop a combination of PZQ and anti-fibrotic drugs in the treatment of murine schistosomiasis which could minimize liver fibrosis simultaneously with worm elimination (Mahmoud et al. 2002).

Silymarin, a standardized milk thistle extract, of which silibinin is the main component, is known for its hepatoprotective and promotes protein synthesis that helps in hepatocyte regeneration (El-Lakkany et al. 2012). This effect could be attributed to its role as an antioxidant, antifibrotic, and antiinflammatory/immunomodulatory agent (Mata-Santos et al. 2010). Mata-Santos et al. (2014) showed that treatment with silymarin leads to a decrease in hepatic granulomas size and to a reduction in liver fibrosis. Their results indicate that silymarin treatment can minimize the deleterious effects of experimental *S. mansoni* infection. Its anti-inflammatory effect could be attributed to its inhibitor role on leukotriene production and on reduction of TNF, IL-1 α , IL-6, IL-2 and IL-4 (Johnson et al. 2002). The aim of this study was to determine cytokine patterns in experimental schistosomiasis *mansoni* infected mice treated with a combination of PZQ and anti-fibrotic drug silymarin.

Materials and methods

Parasite

Cercariae of *S. mansoni* (Egyptian strain) were obtained from experimentally infected *Biomphalaria alexandrina*

snails. These were provided by Schistosome Biological Supply Program Unit (SBSP), Theodor Bilharz Research Institute, Giza, Egypt.

Experimental animals

Hundred laboratory-bred male Swiss albino mice, 8 weeks-old, weighing ~20 g, were obtained from the animal house of the Research Institute of Ophthalmology, Giza, Egypt. They were housed in plastic cages (10 mice/cage) with white wood chips for bedding, fed by commercial complete food mixture and tap water for drinking, and maintained under controlled conditions of lighting (12 h light/12 h dark cycle) and temperature (25 ± 2 °C). Animals were divided into five groups; 20 mice for each group.

Group I (GI)

Healthy uninfected, untreated mice (normal control).

Group II (GII)

Schistosoma mansoni infected untreated mice (infected control). They were infected with 100 ± 10 *S. mansoni* cercariae obtained from infected *B. alexandrina* snails, by the tail immersion method (Liang et al. 1987). Stool examination using a concentration technique was done for each mouse from the 6th week post-infection (PI) to ensure establishment of infection and as a follow up (Kato and Miura 1954).

Group III (GIII)

Schistosoma mansoni infected mice as GII and treated with PZQ (Distocide; EPICO, Egypt). PZQ was given orally in the form of an aqueous suspension in 2 % Cremophore El solution, at a dose of 500 mg/kg body weight divided equally on 2 consecutive days, starting 6 weeks PI (Gonnert and Andrews 1977).

Group IV (GIV)

Schistosoma mansoni infected mice as GII and treated with silymarin (Legalon-Chemical Industries Development; CID, Giza-Egypt, under the license of Madaus GmbH, Germany). Silymarin was given orally in the form of an aqueous suspension in 2 % Cremophore El, at a dose of 140 mg/kg/day, 3 days/week, starting 4 weeks PI (Rabia et al. 2010).

Group V (GV)

Schistosoma mansoni infected mice as GII and treated first by silymarin followed by PZQ.

At 10th and 18th weeks PI, the blood was collected from the mouse's orbital venous plexus (Hoff and Rlagt 2000) of 10 mice from each group. The sera were separated and stored at -20°C until the determination of TGF- β 1, TNF- α , IFN- γ , and IL-4 serum levels. After the mice sacrifice, the livers were removed and fixed in 10 % formalin and processed to paraffin blocks for histopathological studies.

Histopathological studies

For granuloma measurement, microtome sections were cut at a thickness of 5 μm and at a distance of 250 μm apart to avoid re-measurement. Sections were stained with hematoxylin–eosin and Masson's trichrome stain that showed the amount and pattern of collagen formation in the granuloma. The ocular micrometer was used for measurement of the diameter of the liver eggs granulomas (Von Lichtenberg 1962). The mean diameter of each granuloma was obtained by measuring perpendicular diameters using an ocular micrometer. The percent reduction in granuloma diameter relative to the infected control was calculated as follows: % Reduction of granuloma diameter = $\frac{\text{Mean diameter of controls} - \text{Mean diameter of treated groups}}{\text{Mean diameter of controls}} \times 100$. Counting was carried out in five microscopic fields (10×10) in serial tissue sections.

Determination of TNF- α , IFN- γ , IL-4 and TGF- β 1 serum levels

Serum levels of TNF- α , IFN- γ , IL-4 and transforming growth factor- β 1 (TGF- β 1) were estimated by enzyme-linked immunosorbent assays according to the manufacturer's instructions (Quantikine M, R&D systems, Minneapolis, MN, USA for TNF- α ; BioSource International, Inc., Camarillo, California, USA for both IFN- γ and IL-4; Invetrogen Corporation Carlsbad, California, USA for TGF- β 1). These assays employ the quantitative sandwich enzyme immune-assays using one polyclonal antibody and another monoclonal antibody specific for TNF- α , IFN- γ , TGF- β 1 and IL-4. Optical density (OD) values were measured at 450 nm and serum TNF- α , IFN- γ , TGF- β 1 and IL-4 concentrations determined from the standard curves. The sensitivity cutoffs for the ELISA for each of the cytokines were as follows: 10 pg/ml for IL-4 and 20 pg/ml for TNF- α , IFN- γ and TGF- β 1.

Statistical analysis

Collected data were coded and introduced to a PC using the Statistical Package for Social Science (SPSS) for windows version 11.0. Data were represented as the

mean $\pm$ standard deviation (SD) ($n = 10$). The analysis of variance (ANOVA) procedure was used to clarify statistically significant differences between the studied groups. Values were considered statistically significant when $P < 0.05$.

Ethical considerations

The animal experiment was carried out according to the internationally valid guidelines of experimental animal studies and the research protocol was approved by the Institutional Committee of the Faculty of Medicine, Zagazig University. All efforts were made to minimize the animal's suffering.

Results

Results are shown in the Tables 1, 2, 3, 4 and Figs. 1, 2.

Discussion

Granuloma formation and fibrosis are the major causes of morbidity and mortality in association with schistosomiasis. Although the granuloma formation is beneficial for the host because it blocks the hepatotoxic effects of antigen released from parasite eggs, this process may lead to fibrosis with excessive accumulation of collagen and other extracellular matrix proteins in the periportal space (Henri et al. 2002). Cytokines are important regulators of immuno-inflammatory responses and play a major role in the regulation of fibrosis deposition and degradation. Hoffmann et al. (2002) demonstrated the alterations in T helper cytokine profiles during the formation of granulomatous reaction. In this study, the levels of cytokines (IFN- γ , IL-4, TNF- α , TGF- β 1) were measured among the studied groups. Their levels were found to be significantly increased in infected mice compared to normal control. This may attribute to the lack of liver clearance function in hepatic fibrosis that results in increased serum cytokine levels. In the murine model of schistosomiasis, type 1 cytokines such as IFN- γ and activated macrophages have been correlated with immunity. On the other hand, type 2-associated cytokines such as IL-4, IL-13 and IL-10 inhibit classical macrophage activation and have been implicated in granuloma formation and fibrogenesis around tissue-deposited eggs (Morais et al. 2002; Burke et al. 2009).

Regarding the Th-2-related cytokines; it was found that IL-4 showed a highly significant increase in the infected control in comparison to normal control with its peak occurred at 18th week post infection indicating that

Table 1 The mean diameter and number of hepatic granulomas and % of their reduction in *S. mansoni* infected mice treated with PZQ and silymarin either alone or in combination then sacrificed at 10th week PI

Experimental groups (<i>n</i> = 10)	Hepatic granuloma diameter (μm)		Hepatic granuloma number	
	Mean ± SD	Reduction (%)	Mean ± SD	Reduction (%)
Normal control	–	–	–	–
Infected control	327.1 ± 16.5	–	16.4 ± 5.3	–
Infected treated with PZQ	293 ± 14.6 ^a	10.4	11.4 ± 3.1 ^a	30.4
Infected treated with silymarin	290.3 ± 18.6 ^a	11.3	15.3 ± 5.4	6.7
Infected treated with PZQ and silymarin	270.1 ± 12.6 ^a	17.4	9.2 ± 2.7 ^a	43.9

^a *P* < 0.05, statistically significant difference in comparison to infected control**Table 2** The mean diameter and number of hepatic granulomas and % of their reduction in *S. mansoni* infected mice treated with PZQ and silymarin either alone or in combination then sacrificed at 18th week PI

Experimental groups (<i>n</i> = 10)	Hepatic granuloma diameter (μm)		Hepatic granuloma number	
	Mean ± SD	Reduction (%)	Mean ± SD	Reduction (%)
Normal control	–	–	–	–
Infected control	232.3 ± 35.7	–	20.8 ± 7.3	–
Infected treated with PZQ	167 ± 14.6 ^a	28.1	12.3 ± 4.1 ^a	45.6
Infected treated with silymarin	158.3 ± 41.2 ^a	31.8	18.4 ± 2.7	11.5
Infected treated with PZQ and silymarin	101.1 ± 18.4 ^a	56.4	11.1 ± 3.2 ^a	44.7

^a *P* < 0.05, statistically significant difference in comparison to infected control**Table 3** Serum cytokine levels (pg/ml) in mice treated with PZQ and silymarin either alone or in combination then sacrificed at 10th week PI

Experimental groups (<i>n</i> = 10)	TNF-α (pg/ml)	IFN-γ (pg/ml)	IL-4 (pg/ml)	TGF-β1 (pg/ml)
Normal control	90 ± 14.5	205.6 ± 1.70	15.3 ± 0.65	123.36 ± 1.69
Infected control	657.3 ± 72.9 ^a	460.91 ± 14.6 ^a	64.81 ± 8.41 ^a	487.9 ± 2.48 ^a
Infected treated with PZQ	346.6 ± 11.7 ^b	630.6 ± 8.19 ^b	38.4 ± 14.1 ^b	363.44 ± 0.83 ^b
Infected treated with silymarin	436.7 ± 59.1 ^b	562.41 ± 13.4 ^b	41.6 ± 12.8 ^b	281.34 ± 0.78 ^b
Infected treated with PZQ and silymarin	203 ± 26.5 ^b	607.5 ± 14.3 ^b	23.6 ± 13.1 ^b	142.93 ± 0.64 ^b

^a *P* < 0.05, statistically significant difference in comparison to normal control^b *P* < 0.05, statistically significant difference in comparison to infected control**Table 4** Serum cytokine levels (pg/ml) in mice treated with PZQ and silymarin either alone or in combination then sacrificed at 18th week PI

Experimental groups (<i>n</i> = 10)	TNF-α (pg/ml)	IFN-γ (pg/ml)	IL-4 (pg/ml)	TGF-β1 (pg/ml)
Normal control	92.1 ± 16.6	201.65 ± 1.76	19.9 ± 2.94	122.96 ± 0.48
Infected control	927.3 ± 69.6 ^a	350.6 ± 12.6 ^a	97.91 ± 15.61 ^a	986.11 ± 14.3 ^a
Infected treated with PZQ	583.1 ± 22.8 ^b	435.1 ± 1.44 ^b	67.3 ± 7.19 ^b	523.1 ± 0.24 ^b
Infected treated with silymarin	643.6 ± 51.7 ^b	401.3 ± 14.2 ^b	69.6 ± 13.4 ^b	681.8 ± 1.44 ^b
Infected treated with PZQ and silymarin	388.6 ± 26.8 ^b	421.2 ± 0.73 ^b	40.15 ± 1.61 ^b	422.9 ± 0.57 ^b

^a *P* < 0.05, statistically significant difference in comparison to normal control^b *P* < 0.05, statistically significant difference in comparison to infected control

Fig. 1 Photograph of liver sections from mice of different study groups stained with H&E ($\times 100$) 10 weeks PI, **a** Normal liver cell (GI), **b** Liver of infected non treated control group (GII) showing large number of fibrocellular granulomas, **c** Liver of infected mice group treated with PZQ (GIII) showing less number of granuloma, **d** Liver of infected mice group treated with silymarin (GIV) showing decrease in size of granulomas, **e** Liver of infected mice group treated first by silymarin followed by PZQ (GV) showing less number and size of granulomas

Fig. 2 Photograph of liver sections from mice of different study groups, stained with Masson trichrome stain ($\times 100$) 18 weeks PI, showing difference in size of *Schistosoma* granulomas **a** Liver of infected non treated control group (GII) showing large fibrous granuloma, **b** Liver of infected mice group treated with PZQ (GIII) **c** Liver of infected mice group treated with silymarin (GIV) showing decrease in the granuloma size, **d** Liver of infected mice group treated first by silymarin followed by PZQ (GV) showing more reduction in the granuloma size

immune responses to schistosome in chronically infected individuals are typically polarized toward Th2 cytokines. This is characterized by eosinophilia and high levels of IgE (Booth et al. 2004; Abath et al. 2006). At the same time, it decreased in all treated groups compared infected control. It has been suggested that IL-4 upregulates fibroblast chemokine, matrix protein expression, and collagen, which implies that this cytokine is a crucial cytokine for granuloma formation (Liu et al. 2002).

TNF- α is a 26 kDa homotrimeric transmembrane protein that is expressed on the surface of macrophages, T-lymphocytes, natural killer cells, smooth muscle cells, and fibroblasts (Van Deventer 1997). It is an important mediator of murine granuloma formation and hepatic fibrosis. The result of this study showed that silymarin decreased the serum level of TNF- α after it has been markedly increased due to *S. mansoni* infection in mice. In experimental models, Hoffmann et al. (1998) demonstrated that TNF- α exerts a protective effect, whereas other authors attribute to the TNF- α proinflammatory and profibrogenic effects (Henri et al. 2002) that may aggravate the disease.

In the evolution of the granulomatous response to the *S. mansoni* eggs the production of TGF- β 1 may modulate inflammation and regulate fibrogenesis. Several investigators indicated that TGF- β 1 is a regulatory cytokine that is mainly produced by regulatory T cells which provides an effective mechanism of control of the progression of fibrosis in association with IL-10 (Kitani et al. 2003). In the current study, infection of mice with *S. mansoni* caused pronounced elevations in serum TGF- β 1 levels, 10 and 18 weeks PI compared with the control uninfected untreated group. These results were in accordance with Zhu et al. (2000) who reported that the level of serum TGF- β 1 in experimental group was significantly higher than that in control groups at 8, 10, 12 and 16 weeks after infection by schistosomiasis. They added that the level of TGF- β 1 increased with prolongation of infection time. In addition, Kanzler et al. (1999) detected the important role of TGF- β 1 in stellate cell activation and liver fibrogenesis. Moreover, Alves-Oliveira et al. (2006) found that high levels of TGF- β 1 appeared to be associated with protection against fibrosis. Compared to the infected untreated group in this study, treatment with silymarin resulted in a significant reduction in serum TGF- β 1 at 10th and 18th weeks PI. Moreover, the administration of PZQ in combination with silymarin caused normalization with significant reduction of serum TGF- β 1 at 10th and 18th weeks PI, when compared to the corresponding PZQ treated group.

In the present study, there was highly significant difference in the IFN- γ in the infected than in the control group and its peak occurred at 10th week PI then declined after 18th week PI. These results were correlated with Boros (1994) who stated that IFN- γ was found to be

produced very early at the inception of the liver granulomatous response, by the time granulomas reached maximal size where its production declined. Also Silva et al. (2004) agreed with that opinion and suggested that there is marked general downregulation of Th1 responses in infected mice. Caldas et al. (2008) conclude that in the acute phase there is a mixed expression of Th1 and Th2 cytokines with predominance of Th1 in the early infection. The higher production of IFN- γ , in acute as compared to chronic schistosomiasis, partially explain the lack of modulation of the immune response in acute patients. All these results may be due to profound negative effect of parasitic eggs on schistosome antigen driven IFN- γ production. Also, Zhang et al. (1999) reported that the IFN- γ gene transplantation has anti-hepatic fibrosis efficacy in *Schistosoma japonicum*-infected mice, being related to its role of decreasing the expression of TGF- β 1 and TGF- β RII. Moreover Zhang et al. (2004) added that IFN- γ might suppress TGF- β 1 to reverse fibrosis. The mechanism of the suppression is mediated by downregulation of expression of its two receptors at protein level but not by influencing the mRNA expression. On the contrary, Boros and Lukacs (1992) reported that IFN- γ reduces the cellularity of granulomas and downmodulates granuloma size in experimental schistosomiasis. These data are in agreement with de Jesus et al. (2004) that IFN- γ is the major Th1 cytokine involved in the downmodulation of Th2 cells. It was reported that IFN- γ plays a key role in the protective mechanism against periportal fibrosis (Henri et al. 2002).

In this study, the histopathological examination of the livers in group treated with PZQ showed a reduction in the diameter of livers eggs granuloma at all periods of sacrifice compared with infected untreated group in agreement with El-Lakkany and Nosseir (2007). PZQ exert significant regulatory effects on cell immune responses which reduces CD4⁺ T cells and increase CD8⁺ cells resulting in a reduced hepatic granuloma size in schistosomiasis as reported by El-Ahwany et al. (2006). Several studies showed that PZQ is an effective drug in controlling infection with *S. mansoni*, promoting a reduction in egg excretion and regression of fibrosis in mice and humans (Kihara et al. 2007; Martins-Leite et al. 2008). Probably, the increase in T cell reactivity after chemotherapy can be explained by exposure of released antigens to the immune system following the destruction of worms by chemotherapy (Corrêa-Oliveira et al. 2000; Brito et al. 2000).

In addition, there was more decrease in granulomas diameter in both silymarin treated group or combined with PZQ at all periods of sacrifice when compared to infected untreated group. These results agreed with the previous studies (Mata-Santos et al. 2010; Rabia et al. 2010; El-Lakkany et al. 2012). Using Silymarin as an antifibrotics drug can inhibit hepatic stellate cells activation

that subsequently reduced the production of extracellular matrix components and slow down the progression of liver fibrosis (Afdhal and Nunes 2004; Wu et al. 2009). In this study, the obtained reduction in granuloma diameter could be explained by the action of silymarin on the production of pro-inflammatory cytokines/chemokines in hepatic tissue accumulation (Trapoliere et al. 2009; Mata-Santos et al. 2014). Its anti fibrotic effect may attribute to the high levels of IFN- γ and low levels of TNF- α . These results confirmed previously published data that showed that cases of severe portal fibrosis were shown to be associated with low levels of IFN- γ and high levels of TNF- α (Henri et al. 2002; Booth et al. 2004; de Jesus et al. 2004). Indeed, silymarin was found to be able to reduce IL-4 whereas IL-4 was shown to play the role of pro-fibrogenic cytokine (Mata-Santos et al. 2014).

From the obtained results of this study, treatment by PZQ was used in murine schistosomiasis, where specific eradication of the parasite was necessary to enhance the effect of silymarin and the combination of both treatments could reduce hepatic fibrosis by their action on the production of pro-inflammatory cytokines.

References

- Abath FG, Morais CN, Montenegro CE, Wynn TA, Montenegro SM (2006) Immunopathogenic mechanisms in schistosomiasis: what can be learnt from human studies? *Trends Parasitol* 22(2):85–91
- Afdhal NH, Nunes D (2004) Evaluation of liver fibrosis: a concise review. *Am J Gastroenterol* 99(6):1160–1174
- Alves-Oliveira LF, Moreno EC, Gazzinelli G, Martins-Filho AO, Silveira AMS, Gazzinelli A, Malaquias LCC, LoVerde P, Martins Leite P, Correa-Oliveira R (2006) Cytokine production associated with periportal fibrosis during chronic schistosomiasis mansonii in humans. *Infect Immun* 74(2):1215–1221
- Booth M, Mwatha JK, Joseph S, Jones FM, Kadzo H, Ireri E, Kazibwe F, Kemijumbi J, Kariuki C, Kimani G, Ouma JH, Kabatereine NB, Vennervald BJ, Dunne DW (2004) Periportal fibrosis in human *Schistosoma mansonii* infection is associated with low IL-10, low IFN- γ , high TNF- α , or low RANTES, depending on age and gender. *J Immunol* 172(2):1295–1303
- Boros DL (1994) The role of cytokines in the formation of the schistosome egg granuloma. *Immunobiology* 191(4–5):441–450
- Boros DL, Lukacs NW (1992) The role of egg antigens, cytokines in granuloma formation in murine schistosomiasis mansonii. *Mem Inst Oswaldo Cruz* 87(4):75–79
- Brito CF, Caldas IR, Coura Filho P, Corrêa-Oliveira R, Oliveira SC (2000) CD4⁺T cells of schistosomiasis naturally resistant individuals living in an endemic area produce interferon- γ and tumor necrosis factor- α in response to the recombinant 14KDA *Schistosoma mansonii* fatty acid-binding protein. *Scand J Immunol* 51(6):595–601
- Burke ML, Jones MK, Gobert GN, Li YS, Ellis MK, McManus DP (2009) Immunopathogenesis of human schistosomiasis. *Parasite Immunol* 31(4):163–176
- Burke ML, McManus DP, Ramm A, Duke M, Li Y, Jones MK, Gobert GN (2010) Temporal expression of chemokines dictates the hepatic inflammatory infiltrate in a murine model of schistosomiasis. *PLoS Negl Trop Dis* 4(2):e598
- Caldas IR, Campi-Azevedo AC, Oliveira LFA, Silveira AMS, Rodrigo C, Oliveira RC, Gazzinelli G (2008) Human schistosomiasis mansonii: immune responses during acute and chronic phases of the infection. *Acta Trop* 108(2–3):109–117
- Chiaromonte G, Cheever AW, Malley JD, Donaldson DD, Wynn TA (2001) Studies of murine schistosomiasis reveal interleukin-13 blockade as a treatment for established and progressive liver fibrosis. *Hepatology* 34:273–282
- Corrêa-Oliveira R, Rodrigues Caldas I, Martins-Filho OA, Carvalho Queiroz C, Lambertucci JR, Renan Cunha-Melo J, Soares Silveira A, Prata A, Wilson A, Gazzinelli G (2000) Analysis of the effects of treatment of human *Schistosoma mansonii* infection on the immune response of patients from endemic areas. *Acta Trop* 77(1):141–146
- de Jesus AR, Magalhaes A, Miranda DG, Miranda RG, Araujo MI, De Jesus AA, Silva A, Santana LB, Pearce E, Carvalho EM (2004) Association of type 2 cytokines with hepatic fibrosis in human *Schistosoma mansonii* infection. *Infect Immun* 72(6):3391–3397
- El-Ahwany EG, Nosseir MM, Ali LR (2006) Immunomodulation of pulmonary and hepatic granulomatous response in mice immunized with purified lung-stage schistosomulae antigen. *J Egypt Soc Parasitol* 36:335–350
- El-Lakkany N, Nosseir M (2007) Pharmacodynamics of pentoxifylline and/or praziquantel in murine schistosomiasis mansonii. *APMIS* 115(3):184–194
- El-Lakkany NM, Hamman OA, El-Maadawy WH, Badawy AA, Ain-Shoka AA, Ebeid FA (2012) Anti-inflammatory/anti-fibrotic effects of the hepatoprotective silymarin and the schistosomicide praziquantel against *Schistosoma mansonii*-induced liver fibrosis. *Parasit Vectors* 5:9
- Gonnert R, Andrews P (1977) Praziquantel, a new broad-spectrum antischistosomal agent. *Z Parasitenk* 52(2):129–150
- Henri S, Chevillard C, Mergani A, Paris P, Gaudart J, Camilla C, Dessein H, Montero F, Elwali NE, Saeed OK, Magzoub M, Dessein AJ (2002) Cytokine regulation of periportal fibrosis in humans infected with *Schistosoma*: IFN- γ is associated with protection against fibrosis and TNF- α with aggravation of disease. *J Immunol* 169(2):929–936
- Hoff J, Rlagt LV (2000) Methods of blood collection in the mouse. *Lab Anim* 29(10):47–53
- Hoffmann KF, Caspar P, Cheever AW, Wynn TA (1998) IFN γ , IL-12, and TNF- α are required to maintain reduced liver pathology in mice vaccinated with *Schistosoma mansonii* eggs and IL-12. *J Immunol* 161(8):4201–4210
- Hoffmann KF, Wynn TA, Dunne DW (2002) Cytokine mediated host responses during schistosome infections: walking the fine line between immunological control and immunopathology. *Adv Parasitol* 52:265–307
- Johnson VJ, Osuchowski MF, He Q, Sharma RP (2002) Physiological responses to a natural antioxidant flavonoid mixture, silymarin, in BALB/c mice: II. Alterations in thymic differentiation correlate with changes in c-myc gene expression. *Planta Med* 68(11):961–965
- Kanzler S, Lohse AW, Keil A, Henninger J, Dienes HP, Schirmacher P, Rose-John S, Zum Büschenfelde KHM, Blessing M (1999) TGF- β 1 in liver fibrosis: an inducible transgenic mouse model to study liver fibrogenesis. *Am J Physiol Gastrointest Liver Physiol* 276(2):1059–1068
- Kato K, Miura M (1954) On the comparison of some stool examination methods. *Jpn J Parasitol* 3:35
- Kihara JH, Muhoho N, Njomo D, Mwobobia IK, Josyline K, Mitsui Y, Awazawa T, Amano T, Mwandawiro C (2007) Drug efficacy of praziquantel and albendazole in school children in Mwea division, Central Province, Kenya. *Acta Tropica* 102(3):165–171

- Kitani A, Fuss I, Nakamura K, Kumaki F, Usui T, Strober W (2003) Transforming growth factor (TGF- β 1) producing regulatory T cells induce Smad-mediated interleukin 10 secretion that facilitates coordinated immunoregulatory activity and amelioration of TGF- β 1 mediated fibrosis. *J Exp Med* 198(8):1179–1188
- Kresina TF, He Q, Zern MA (1992) Cytokines, hepatic fibrosis and schistosomiasis. *R I Med* 75:191–195
- Liang Y-S, Bruce JI, Boyd DA (1987) Laboratory cultivation of schistosome vector snails and maintenance of schistosome life cycles In: Proceedings of the first Sino-American symposium, Santa Cruz, pp 34–48
- Liu X, Kohyama T, Wang H, Zhu YK, Wen F-Q, Kim HJ, Romberger DJ, Rennard SI (2002) Th2 cytokine regulation of type I collagen gel contraction mediated by human lung mesenchymal cells. *Am J Physiol* 282(5):L1049–L1056
- Mahmoud MA, El-Shafei MA, Mttá R, Hany AA (2002) Parasitological and immunological changes in murine hepatic schistosomiasis before and after praziquantel treatment. *J Egypt Soc Parasitol* 32(2):551–560
- Martins-Leite P, Gazzinelli G, Alves-Oliveira LF, Gazzinelli A, Malaquias LC, Correa-Oliveira R, Teixeira-Carvalho A, Silveira AM (2008) Effect of chemotherapy with praziquantel on the production of cytokines and morbidity associated with schistosomiasis mansoni. *Antimicrob Agents Chemother* 52(8):2780–2786
- Mata-Santos HA, Lino FG, Rocha CC, Pyrrho SA (2010) Silymarin treatment reduces granuloma and hepatic fibrosis in experimental schistosomiasis. *Parasitol Res* 107(6):1429–1434
- Mata-Santos HA, Dutra FF, Rocha CC, Lino FG, Xavier FR, Chinalia LA, Hossy BH, Castelo-Branco MT, Teodoro AJ, Paiva CN, dos Santos Pyrrho A (2014) Silymarin reduces profibrogenic cytokines and reverses hepatic fibrosis in chronic murine schistosomiasis. *Antimicrob Agents Chemother* 58(4):2076–2083
- Morais CNL, Souza JR, Melo WG, Aroucha ML, Domingues ALC, Wynn T, Abath FGC, Montenegro SML (2002) Studies on the production and regulation of interleukin, IL-13, IL-4 and interferon- γ in human schistosomiasis mansoni. *Mem Inst Oswaldo Cruz* 97(1):113–114
- Rabia I, Nagy F, Aly E, Mohamed A, El-Assal F, El-Amir A (2010) Effect of treatment with antifibrotic drugs in combination with PZQ in immunized *Schistosoma mansoni*-infected murine model. *J Am Sci* 6:208–216
- Silva LM, Menezes RM, De Oliveira SA, Andrade ZA (2003) Chemotherapeutic effects on larval stages of *Schistosoma mansoni* during infection and reinfection of mice. *Rev Soc Braz Med Trop* 36(3):335–341
- Silva LM, Oliveira SA, Ribeiro-dos-Santos R, Andrade ZA, Soares MBP (2004) Comparison of immune responses of *Schistosoma mansoni*-infected mice with distinct chronic forms of the disease. *Acta Trop* 91(2):189–196
- Trapoliere M, Caligiuri A, Schmid M, Bertalani C, Failli P, Vizzutti F, Pinzani M (2009) Silybin, a component of silymarin, exerts anti-inflammatory and antifibrogenic effects on human hepatic stellate cells. *J Hepatol* 50(6):1102–1111
- Utzinger J, Keiser J (2004) Schistosomiasis and soil-transmitted helminthiasis: common drugs for treatment and control. *Expert Opin Pharmacother* 5(2):263–285
- Van Deventer SJ (1997) Tumor necrosis factor and Crohn's disease. *Gut* 40(4):443–448
- Von Lichtenberg FC (1962) Host response to eggs of *Schistosoma mansoni*. I Granuloma formation in the unsensitized laboratory mouse. *Am J Pathol* 41(6):711–731
- Williams DL, Asahi H, Botkin DJ, Stadercker MJ (2001) Schistosome infection stimulates host CD4⁺ T helper cell and B-cell responses against a novel egg antigen, thioredoxin peroxidase. *Infect Immun* 69(2):1134–1141
- Wu JW, Lin LC, Tsai TH (2009) Drug-drug interactions of silymarin on the perspective of pharmacokinetics. *J Ethnopharmacol* 121(2):185–193
- Zhang L, Yao H, Cao X, Yu Y, Chen H, Li M (1999) Effects of IFN- γ gene-modified hepatocytes on TGF- β 1 and its receptor in mice infected with *Schistosoma japonicum*. *Zhongguo Ji Sheng Chong* 17(6):330–333
- Zhang BB, Jiao YW, Cai WM, Tao J, Liu RH (2004) Influence of interferon gamma treatment on expression of TGF- β 1 and its receptors in liver fibrosis of mice with schistosomiasis japonica. *Xue Yu Ji Sheng Chong* 22(6):340–343
- Zhu H, Zeng L, Zhu D, Yuan Y (2000) The role of TGF- β 1 in mice hepatic fibrosis by schistosomiasis japonica. *J Tongji Med Univ* 20(4):320–329